Morphometric Changes Due To β Carotene on Wistar Rats Fed Dietary Fat

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Abstract: Beta-carotene (βC), an antioxidant found in fruits and vegetables is responsible for the yellow color of these plants. The aim of the present research was to investigate the morphometric changes on Wistar rats fed high dietary fat and βC. Thirty (30) male Wistar rats were randomly grouped into 6. Group A was the Control and received distilled water, Group B received high-fat diet of 60% fat and 40% rat chow, Group C received 300mg/kg body weight (BW) of βC, Group D received high-fat diet for 12 weeks and was treated with 300mg/kg BW of βC for 2 weeks, Groups E received 300mg/kg BW of βC for 2 weeks and then received high-fat diet for12 weeks while Group F received high-fat diet for 12 weeks and was treated with 150mg/kg BW of βC for 2 weeks. At the end of 16 weeks, the weights, head-tail lengths were measured and the animals humanely sacrificed, the weights of the liver measured. The results showed that increase in high-fat diets did not sequel increase in the body weight and liver weight in the treated groups. When compared to Groups D and F, Group C showed increased body and liver weights but these were lower than that observed in Group A. The liver weight was increased in animals feed dietary fat alone when compared to Group A. The result showed that Lee index was higher in Groups D and F when compared to Groups B and E. The results of the present study suggest that β carotene extract can be very effective in treating weight gain due to high fat diets.

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1. Introduction:

Dietary fat intake often has been shown to be responsible for the increase in adiposity. Human studies have shown that high-fat diets (≥30% of energy from fat) can easily induce obesity (French and Robinson, 2003). Epidemiological studies have shown that when the average amount of fat in the diet increases, the incidence of obesity also increases (Saris *et al*., 2000).

This has led to a worldwide effort to decrease the amount of fat in human diets. Diets rich in fat induce obesity in humans and animals (Buettner *et al*., 2007). In both rats and mice, a positive relationship has been found between the level of fat in the diet and body weight (Ghibaudi *et al*., 2002; Takahashi *et al*., 1999). It was shown that rats consuming diets containing high proportions of fat gained weight faster than those on diets containing minimal amounts of fat (Buettner *et al*., 2007). Obesity was induced for the first time in rats by *ad libitum* feeding of a semi-liquid palatable diet (Ingle, 1949).

Fenton and Dowling (1953) used high-fat diets with fat at 50% of total energy in weaning mice to induce nutritional obesity but the model was later renamed dietary obesity (Sclafani & Springer, 1976). Animal models have been widely used for experiments on dietary obesity (Speakman *et al.,* 2007). Usually, high-fat diets within the range of 30–78% of total energy intake were used either by adding a particular fat to the diet or using an assortment of fat and sugar-rich supermarket foods for studying obesity in rats and mice (Woods *et al*., 2003; Huang *et al*., 2004). The use of high carbohydrate low-fat diets has not been found as efficient as high fat low carbohydrate diets in inducing obesity (Ghibaudi *et al*., 2002).

It has been reported that despite the growing problem of obesity, Canadians and Americans are eating more fat than a generation ago (Lissner *et al*., 2000). This showed that the increasing rate of obesity cannot be totally explained by high fat in the diet, suggesting that the type of fat may also play a role (Moussavi *et al.,* 2008). Some studies have reported that not all fats are obesogenic and the dietary fatty acid profile rather than the amount of energy from fat is an important variable in developing dietary obesity (Kien *et al*., 2005). There are reports of non-significant differences in the final body weight and body weight gain of the animals consuming various fatty acids (Ellis *et al*., 2002).

Other factors that may contribute to obesity induced by a diet rich in fat include failure to adjust oxidation of fat to the extra fat in the diet, increase in adipose tissue

lipoprotein lipase activity, increased meal size and decreased meal frequency as well as over-consumption of energy attributed to high energy density of the diets (Buettner *et al*., 2007). The aim of the present study was to evaluate the morphometric changes on Wistar rats fed high dietary fat and beta-Carotene.

2. Methodology:

2.1. Preparation of the extract

Fresh carrots were purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The carrots were dried under shade for three weeks and were grounded into powder. The pulverized carrots were wrapped with Whatman filter paper and placed into the chamber of the Soxhlet extractor. Then, 250ml of N-Hexane was added to the Soxhlet flask and placed on a heating mantle. The solvent was heated at 50^0C, the Soxhlet extractor condenses the sample in the filter paper and the content of the carrots were extracted until clear solvent started coming out of the extraction chamber. The extract was concentrated using Water bath at 50^0C and was then stored in the refrigerator.

2.2 Animal Procurement

Thirty (30) Male Wistar rats of the average weight of 71.0g were procured from and were maintained in the animal house of the Department of Biological Science, Federal University Ndufu-Alike Ikwo, Ebonyi State Nigeria. The animals were housed in metal cages, fed and water was allowed *ad libitum* with acclimatization period of two weeks.

2.3. High Fat Diet preparation

Cow fat was purchased from Meat Market Abakaliki, Ebonyi State, Nigeria. The fat was dissolved by heating, collected in metal containers and stored in the refrigerator. High Fat Diet was prepared by mixing 60% of cow fat and 40% of normal rat chow as described by Ghibaudi *et al.* (2002) and then was stored in the refrigerator.

2.4. Animal Experimentation

The rats used were randomly divided into 6 groups. Group A received normal rat chow for 14 weeks. Group B received high-fat diet daily for 14 weeks. Group C received 300mg/kg body weight (BW) of β-Carotene daily for 14 weeks. Group D received High-fat diet (HFD) daily for 12 weeks and then 300mg/kg BW of β-Carotene daily for 2 weeks. Group E received 300mg /kg BW of β-Carotene daily for 2 weeks, and then HFD daily for 12 weeks. Group F received HFD daily for 12 weeks and then 150mg/kg BW of β-Carotene daily for 2 weeks.

2.5. Assessment of weight and obesity:

The weights and Naso-anal lengths of the rats in each group were measured weekly. At the end of the experiment, all the animals were weighed and Naso-anal lengths measured. The weights were used to calculate the change in weight while the Naso-anal lengths were used to calculate the Lee Index (LI) which is the cube root of body weight divided by the Naso-anal length multiplied by 1000 as was described by Lee (1929) and Xu *et al. (*2010).

2.6. Assessment of Liver Index

At the end of the experiment, the animals were anesthetized using ketamine and were sacrificed. Then a mid-line incision was made through which the livers were removed. The weights of the livers were measured and were used to calculate the Liver weight index (LWI in %) which was calculated as the liver weight divided by the body weight multiplied by 100 as described by el-Din *et al.* (2015).

2.7. Data Analysis

Data obtained were expressed as mean ± SD. The level of homogeneity among the groups was tested using one-way Analysis of Variance (ANOVA). Where heterogeneity occurred the groups were separated using Duncan Multiple Range Test. A value of $p < 0.05$ was considered significant using Statistical Package for Social Sciences (version 20.0).

3. Results

The result of the present study showed that the body weight gain in the Control animals (Group A) was significantly increased compared to the animals in all the experimental groups ($P<0.05$). However, the weight gain in animals in Groups C, D, E and F had a significant increase when compared to the animals in Group B while the weight gain in animals in Group C had a significant weight gain when compared to animals in Groups B, D, E and F (Table 1). Meanwhile, there was a significant decrease in the weight gain of the animals in Group C when compared to the Control animals (Group A) ($P<0.05$) as shown in Table 1.

Table 1: *Effect of HFD and βC on the Body weight.*

Group	Initial weight (g)	Final weight (g)	Weight gain (g)	% weight gain
A	78.37 ± 12.61	215.94 ± 32.14	137.57	175.54*
B	76.73 ± 13.34	88.37 ± 5.10	11.64	15.17
C	71.11 ± 8.07	170.90 ± 9.56	99.79	140.33*
D	74.59 ± 12.40	127.18 ± 8.63	52.59	70.51**

E	54.45 ± 4.72	86.99 ± 27.80	32.54	59.67*
F	65.18 ± 1.65	129.52 ± 9.78	64.34	98.71**

Values expressed as Mean ± SD; N=5, *P<0.05; and % Weight Gain=weight gain/initial weight x100

The result from Table 2 showed that increased consumption of the Fat-Diet in the animals in Group B was not commensurate with the increase in weight gain. However, there was a significant decrease in the weight gain of the animals in Group B when compared to Groups A, C, D, E and F (P<0.05).

Table 2: *The effect of total fat diet consumed on the % weight gain.*

Group	Total fat diet consumed	% Fat diet consumed	Weight gain (g)	Weight gain %
A	00	00	137.57	175.54
B	3100.68	27.89	11.64*	15.17
C	00	00	99.79	140.33
D	2590.35	23.30	52.59	70.51
E	2730.02	24.56	32.54	59.67
F	2696.64	24.26	64.34	98.71

% Fat diet consumed =total fat diet consumed per group for 12weeks/total fat diet consumed by all groups for 12weeks x100.

The result from Table 3 showed a significant increase in Lee index of the animals in the Group A when compared to the animals in Group B (P<0.05). However, there was a significant decrease in the Lee Index of animals in Group B when compared with the animals in Groups A, C, D, E and F (P<0.05) as shown in Table 3.

Table 3: Effect of HFD and βC on the Lee Index

Group	Body Weight (g)	Naso-anal Length (cm)	Lee Index (LI)
A	215.94 ± 32.14	20.44 ± 1.09	293.51 ± 3.10
B	88.37 ± 5.10	16.25 ± 0.07	271.10 ± 4.09*
C	170.90 ± 9.56	18.92 ± 0.33	293.31 ± 1.44
D	127.18 ± 8.63	16.60 ± 0.36	302.95 ± 3.68
E	86.99 ± 27.80	15.35 ± 1.34	288.66 ± 6.60
F	129.52 ± 9.78	16.50 ± 0.42	306.64 ± 0.21

Values are expressed as Mean ± SD; N=5; *P<0.05 and Lee Index= cube root of Body-weight/Naso-anal length x1000.

The result from Table 4 showed a significant increase in the liver index of the animals in Group B when compared with those in the Groups A, C, and D (P<0.05). While there was a significant decrease in the Liver index of the animals in Group C when compared with the animals in Groups A, B, and D when compared with those animals in Group C (P<0.05) as shown in Table 4.

Table 4: *Effect of HFD and βC on Liver Weight Index (LWI).*

Group	Body weight (g)	Liver weight (g)	Liver Index
A	215.94 ± 32.14	7.35 ± 0.39	3.40 ± 0.12*
B	88.37 ± 5.10	3.45 ± 0.001	3.90 ± 0.01
C	170.90 ± 9.56	5.10 ± 0.51	2.98 ± 0.53*
D	127.18 ± 8.63	4.65 ± 0.0	3.66 ± 0.35*
E	86.99 ± 27.80	3.45 ± 0.64	3.97 ± 0.23
F	129.52 ± 9.78	4.95 ± 0.10	3.82 ± 0.01

Values expressed as Mean ± SD; N=5; *P<0.05 and Liver Index= Liver weight/Body-weight x100.

4. Discussion

Dietary fats are important sources of essential fatty acids (Aranceta, & Pérez-Rodrigo, 2012) and it has been reported that an uncontrolled intake of dietary fats could lead to obesity, type 2 diabetes mellitus, dyslipoproteinaemia, hypertension and metabolic syndrome including coronary heart disease, stroke, and cancer (Wolfram *et al*., 2015).

In animal models, as in humans, obesity can be assessed by criteria based on gain of body weight (WHO, 2005) or the Lee obesity index and/or an increase of body fat content (Ichihara & Yamada, 2008). However, standard thresholds for obesity have not been developed like BMI in human beings. In most studies, the degree of obesity has been evaluated by comparing body weight or fat of the experimental group fed a high-fat or energy-dense diet with control animals that show normal growth while fed chow or low-fat diets (Ghibaudi *et al*., 2002; Woods *et al*., 2003). Researchers that have attempted to do so and concluded that the difference in the values that are 10–25% greater body weight than age-matched control rats fed chow (normal pattern of body-weight gain) as moderate obesity (Wardi *et al*., 2001; Woods *et al*., 2003) and greater than 40% as severe obesity (Levin *et al.,* 2002; Kiess *et al.,* 2008).

The Lee index for assessing obesity in rats is similar to BMI in humans. It was defined by Lee (1929) as the cube root of body weight (g) divided by the Naso–anal length (cm) and multiplied by 1000. Lee considered values greater than 310 as an indicator of obesity. Since then some researchers have used the Lee index to assess the levels of obesity in rats (Sclafani & Gorman, 1977). Reliable correlations were found in some studies between the Lee index and fat content of the body (Hariri & Thibault, 2010).

The results from the present study showed that increased high-fat diet at the end of 12weeks did not show an increase in the body and liver weight in the treatment groups, although the increase in the body weight was

recorded in weeks 13 and 14 for the treatment groups although the weight of the control animals was increased every week. This might be due to a metabolic imbalance of carbohydrate, protein, and fat. These results were not in consonance with the report given by el-Din *et al*. (2015), who concluded that dietary fat intake causes weight gain throughout the period of the experiment. The result also showed an increase in body and liver weights of the experimental animals which were lower than that observed in the Control. Liver weight was increased in animals fed with dietary fat alone for 12 weeks, but this was decreased in the animals in the treatment group when compared to the control. This could mean that β-Carotene acts by decreasing fat accumulation in the liver and thereby decreasing the liver index.

The Lee index is higher in group D and F when compared to Groups B and E, while the value of Group C and A was not significant. However, the decrease in the body weight and Lee Index was reversed after the administration of beta-carotene for 2 weeks and as such there was an inverse relationship between fat consumption and weight gain as revealed from the present study.

5. Conclusion

It could be right to conclude that diet-induced obesity is dependent on the dietary sources as results from the present study suggested that the experimental animals were not obsessed. The liver weight index suggested that despite that the animals were not obsessed, the liver showed evidence of damage as a result of the high dietary fat consumption while beta-carotene has the potential to revert the changes caused by the dietary fats.

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